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Reduced hepatocellular expression of canalicular transport proteins in infants with neonatal cholestasis and congenital hypopituitarism

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List of abbreviations

ACTH	adrenocorticotropic hormone
AST	aspartate aminotransferase
BSEP	bile salt export pump
CEs	canalicular ectoenzymes
CTPs	canalicular transport proteins
FIC1	familial intrahepatic cholestasis type 1
GGT	γ-glutamyltransferase
GH	growth hormone
HP	congenital hypopituitarism
SOD	septo-optic dysplasia
TSH	thyroid stimulating hormone

Objective: To assess whether prolonged neonatal cholestasis, described in congenital hypopituitarism (HP) and septo-optic dysplasia (SOD), is associated with altered expression of selected canalicular ectoenzymes (CEs) and canalicular transport proteins (CTPs).

Study design*:* Children with HP (n=21), SOD (n=18), and cholestasis seen in our centre over 26 years were reviewed. Histopathologic findings in archival liver-biopsies were assessed (n = 10) and in those with low/normal levels of serum γ-glutamyltransferase (GGT) activity despite conjugated hyperbilirubinaemia, expression of CEs and CTPs was evaluated immunohistochemically.

Results: Patients presented at a median age of 8 weeks [range, 3-20] with median total bilirubin 116 µmol/L [45-287], GGT 95 IU/L [25-707] and serum cortisol 51 nmol/L [17-240]. All but 3 had low free thyroxin [median 9.6 pmol/L (6.8-26.9)] with raised thyroid stimulating hormone levels [median 5.95 mU/L (<0.1-9.24)]. Liver histological features included moderate to severe intralobular cholestasis with non-specific hepatitis, giant-cell transformation of hepatocytes and fibrosis. In all, immunohistochemical staining for CEs and CTPs revealed a degree of reduced expression, associated with normal serum GGT values in 6 of the 10 patients, and another 6 non-biopsied cholestatic infants also had low/normal serum GGT activity. Sequencing of *ABCB11* and *ATP8B1* performed in six of the biopsied patients did not identify pathogenic mutations. Following replacement therapy biochemical evidence of hepatobiliary injury resolved in all children within a median period of 6 months.

Conclusion: Hepatobiliary involvement in HP associated with SOD has a good prognosis, but its aetiology remains uncertain. Immunohistochemical expression of CTPs was reduced in available liver samples.

The association of liver dysfunction with hypopituitarism (HP) was first suggested by de Mosier in 1956 [1]. Several series of patients with congenital HP, with or without septo-optic dysplasia (SOD), and mixed (conjugated and unconjugated) hyperbilirubinaemia have been described [2-9]. Reported liver biopsy findings in these patients are non-specific, with intralobular cholestasis, mild portal inflammation, and/or giant-cell transformation of hepatocytes. Following replacement therapy with hydrocortisone and often thyroxin, clinical and biochemical evidence of hepatobiliary injury typically resolves. Failure to initiate early treatment can exceptionally lead to cirrhosis and subsequent liver transplantation [9]. The causal relationship between endocrine abnormalities and cholestasis, however, remains poorly understood.

Clinical features suggestive of HP include hypoglycaemia, hypothermia, ambiguous genitalia and electrolyte imbalance. "Soft" dysmorphic facial features (frontal bossing, depressed nasal bridge and saddle-shaped nose) are described [9, 10]. Reported ocular abnormalities in SOD vary from poor visual acuity to complete blindness due to optic nerve dysplasia or atrophy. Brain defects associated with SOD include hypophyseal hypoplasia and midline brain abnormalities, such as absence of the corpus callosum and/or septum pellucidum [11].

Some of our cholestatic patients with HP had serum γ-glutamyltransferase (GGT) activity levels either normal or disproportionately low for the degree of conjugated hyperbilirubinaemia present. The canalicular transport protein (CTP) bile salt export pump (BSEP) and several canalicular ectoenzymes (CEs) may be abnormally expressed in various forms of intrahepatic cholestasis that are associated with normal-range ("low") GGT activity. We hypothesised that canalicular antigens might be expressed abnormally in HP.

The clinical phenotypes described in the previous series, owing to mutation in *ATP8B1* or *ABCB11,* vary substantially within and between familial intrahepatic cholestasis type 1 (FIC1) deficiency

and BSEP deficiency. They range from mild, episodic cholestasis and pruritus ("benign" recurrent intrahepatic cholestasis, or BRIC) to end-stage chronic liver disease requiring liver transplantation [12-15]. Clinical variation occurs even among patients with the same mutations, suggesting that factors other than the mutations themselves may influence expression or function of CEs and CTPs, as observed with transient neonatal cholestasis [16, 17] or perturbations in hormonal milieu (pregnancy, oral-contraceptive use) that could precipitate cholestasis in previously asymptomatic individuals, who harbour polymorphisms in *ATP8B1* or *ABCB11* [18, 19].

We thought it was conceivable that *ATP8B1* or *ABCB11* variation might conduce to intrahepatic cholestasis in HP. Accordingly, we immunohistochemically evaluated CE and CTP expression, in the patients with HP and normal ("low") serum GGT activity despite cholestasis.

METHODS

Approximately 150 infants with liver disease are referred annually to the tertiary-care paediatric liver centre at King's College Hospital, London. A retrospective analysis of the patient database from 1990-2016 was undertaken to identify children with neonatal cholestasis associated with HP.

Infants referred for investigation of prolonged conjugated jaundice undergo clinical, biochemical, and radiological evaluation, often including percutaneous liver biopsy. Initial endocrine investigations include determinations of random serum concentrations of glucose, cortisol, thyroid stimulating hormone (TSH), and free thyroxin. Children with random serum cortisol values <100 nmol/L routinely undergo short adrenocorticotropic hormone (ACTH; "Synacthen") testing (intramuscular injection of 62.5 µg followed by measurement of cortisol levels at 0, 30, and 60 minutes) and detailed ophthalmologic examination for signs of SOD. If findings on ACTH testing are abnormal, cranial magnetic resonance imaging [20] is performed to look for anatomic abnormalities of the pituitary gland and optic nerves.

Percutaneous liver biopsy is performed when the aetiology of cholestasis cannot be established on the basis of non-invasive tests. Normal-range or "low" GGT activity in some infants prompts immunohistologic study of expression of CTPs and CEs, including GGT, in the liver biopsy material. In the present work, immunohistologic evaluation was performed retrospectively (Table 1).

Archived liver specimens were available from 10 patients. Tissue sections cut at 4μm were stained with haematoxylin-eosin and, after diastase digestion, with periodic acid-Schiff technique. In addition, parallel sections were immunostained (DAKO, Chem-Mate, Ely, UK) with a privately generated rabbit polyclonal antibody against a peptide with the sequence of C-terminal BSEP [21] and with mouse monoclonal antibodies against epitopes of the homologous ATP-binding cassette canalicular transporters MRP2 (Signet/Bioquote, York, UK; clone M2III6) and MDR3 (Alexis Biochemicals ALX-801-028, Nottingham, UK) as well as for the ectoenzymes alanyl aminopeptidase (CD13; Novocastra NCL-CD13-304, mouse monoclonal, clone 38C12; Vector Laboratories, Newcastle-upon-Tyne, UK) and GGT (Abnova H00002678-M01, mouse monoclonal, clone 1F9; Novus Biologicals, Cambridge, UK). All commercial products were used according to manufacturers' instructions.

Stored consented blood samples were available for 6 patients. DNA was extracted from stored leucocyte-pellet samples (-80^{o}C). Sanger sequencing was performed for all exons, with exon-intron junctions, of *ABCB11* [22] and *ATP8B1* [20].

RESULTS

Twenty-one patients (12 male, 57%) with the diagnosis of HP were identified. All patients were born by spontaneous vaginal delivery; all but 2 were born at term (patients 9 and 13 at 27 and 31 weeks' gestation, respectively). Median birth weight was 2.2 kg [range, 0.975-4.3] The antenatal history was unremarkable in all, apart from patient 13 whose mother had abused cocaine and who developed neonatal abstinence syndrome. Median age at admission for evaluation of prolonged conjugated

jaundice was 8 weeks [range, 2-20]. Seven (33%) children were referred during the first month of life. Percutaneous liver biopsy was performed in 10 children. None of the patients had a family history of endocrine disorder, consanguinity, or developmental problems. All other causes of prolonged neonatal conjugated hyperbilirubinaemia were excluded.

Clinical findings

Patients 9, 13, and 20 had micropenis, and frontal bossing was evident in patients 9, 14, and 18. Patients 1, 7-9, 15, and 20 presented with recurrent hypoglycaemic episodes. Median serum total bilirubin concentration at initial evaluation in our institution was 116 µmol/L [range 45-287] with a median conjugated fraction of 83 µmol/L [range 32-165]. All infants had transiently raised serum aspartate aminotransferase (AST) activity, with a median level of 154 IU/L [range 66-445 IU/L, nv <50 IU/L]. The median GGT value was 95 IU/L [range 25-707 IU/L, nv <55 IU/L]; 12 patients (57%; Table 2) had normal serum GGT activity for age and sex [23].

All patients except 7, 10, 17, and 21 had abnormal random serum cortisol concentrations, median 51 nmol/L (range, 18-240; Table 3). These 4 infants with normal baseline serum cortisol values had abnormal thyroid function tests and thus went on to undergo ACTH testing. All but patients 4, 15, and 16 had abnormal serum TSH concentrations. Twelve of these patients had low serum free thyroxin concentrations.

Magnetic resonance imaging assessment of the pituitary gland and optic nerves was performed in all patients except 5 and 12. Among the 19 patients who underwent brain imaging, all but 2 (1, 10) had pituitary gland abnormalities. In some (n=3), the pituitary stalk was absent, in some (n=2), the sella turcica was small and the pituitary fossa was shallow, optic nerves were atrophic (n=11) and the neurohypophysis was small (n=13). Some patients had more than one abnormality. Imaging in patient 10 was normal. In patient 1 the anterior pituitary appeared normal, but ectopic pituitary tissue lay

dorsal to the optic chiasm. All patients underwent ophthalmologic examination, with dysplastic or aplastic changes of the optic disc diagnosed in 11 (52%).

The 18 patients, diagnosed with HP and hypothyroidism, were started on treatment with hydrocortisone (2.5 mg 3 times/day) and thyroxin (25 μg/day). Three patients with normal thyroid function were given only hydrocortisone (2.5 mg 3 times/day). In the second year of life, growth hormone (GH) supplementation therapy (25 μg/kg/day) was started in 14 of the 21 patients because of poor linear growth. The median time for serum AST and bilirubin levels to normalise was 6 months (range, 3-9). None of the patients had clinical or ultrasonographic signs of liver disease during follow-up and no further liver biopsies were performed.

The liver biopsy findings included moderate to severe degrees of cellular and canalicular cholestasis in all cases, with prominent multinucleated giant cell transformation in most of them. The portal tracts were mildly expanded due to mixed inflammatory cell infiltrates and fibrosis, while the parenchyma had occasional haemopoietic foci, patchy hepatocyte necrosis, marked siderosis of macrophages and some scattered fatty vacuoles. (Table 1)

On immunostaining, CD13 was expressed at apices of cholangiocytes and along canalicular margins. Expression of MRP2 was reduced in patients 5 and 8 and less widespread than that of CD13 (Figure 1). There was markedly reduced expression of GGT in 5/7 patients. Expression of BSEP was present along the canaliculi (Figure 1), but in all patients it was only focal and was less strong than in age-matched controls (Table 3). MDR3 immunostaining was present along canalicular margins, although its expression was considerably weaker and less widespread than that of MRP2 except in patients 5 and 8. Controls marked appropriately.

In view of the reduced expression of CTPs and GGT on immunostaining, genetic screening was undertaken to exclude a genetic cause of cholestasis. Sanger sequencing of all exons of *ABCB11* and

ATP8B1, performed on patients 1, 3, 4, 7, 8, and 10, in whom DNA was available, identified no mutations.

DISCUSSION

We note that 57 % of these cholestatic infants with primary HP have low or normal serum GGT. We infer that HP could underlay intrahepatic cholestasis, rather than simply co-existing with it, from observed resolution of cholestasis with corticosteroid and thyroxin therapy. Our findings suggest that cortisol and thyroxin may be required for normal hepatocellular expression of CTPs such as BSEP, MDR3, and MRP2 as well as of CEs such as GGT.

Our series documented an incidence of HP in 1% of infants with conjugated hyperbilirubinaemia referred to a specialised paediatric hepatology centre. This prevalence is lower than the 5% reported in general paediatric single-centre series [2, 24]. The incidences of HP and SOD in the general paediatric population are reported as 1/100,000 [25] and 10.9/100,000 [26], respectively. Infants with primary adrenal insufficiency vary in severity of cholestasis, although isolated ACTH deficiency (secondary hypoadrenalism) has not been documented in cholestatic patients [2, 5].

We have observed a slight male prevalence in children with SOD from our series, as eight (73%) of 11 infants with ocular involvement were boys, whereas among the remaining 10 only 4 (40%) were male. In similar series 4 of 8 patients [9] and 3 of 5 [4] patients with SOD were male.

Once supplementation with hydrocortisone and thyroxin was initiated, liver impairment started to improve in all patients within the first nine months of life. Spray et al. reported that biochemical indices of hepatobiliary injury normalised in 7 of 12 infants within 6 weeks of initiating such supplementation [9]. We could not identify any difference between the HP children with and without SOD

in respect of severity of liver disease or length of time required to clear jaundice.

Fourteen patients in our series required growth hormone in the second year of life when biochemical signs of liver dysfunction had already disappeared. This contrasts with findings in some previous studies [11, 24], including an early report describing one infant, diagnosed in the neonatal period with growth hormone deficiency, who despite treatment with hydrocortisone had ongoing liver injury on liver biopsy at age 2 years, demonstrating prominent fibrosis and a micronodular distortion of lobular architecture [5]. It may be speculated that this infant had, in addition to growth hormone deficiency, another underlying liver disorder, not appreciated at the time.

Histological signs of liver injury in our patients were relatively mild and non-specific and included hepatocellular and canalicular cholestasis, mild portal-tract inflammation, and giant-cell transformation of hepatocytes. As subsequent biopsies were unjustified in the absence of clinical and biochemical signs of liver disease we can only speculate that the initial histological damage was temporary. Minami et al. have described a 7-year-old girl with HP and SOD in whom liver biopsy findings were interpreted as congenital hepatic fibrosis [27]. This may have been co-incidence; none of our 10 biopsied patients had histological features of ductal plate malformation and none of our 21 patients has clinical signs of chronic liver disease or portal hypertension during follow-up.

Some infants with conjugated hyperbilirubinaemia have serum GGT levels that are either normal or inappropriately low. Some such patients harbour mutations in *ATP8B1*, encoding an enzyme that shifts aminophospholipids from the external to the internal hemi-leaflet of the bile-canaliculus membrane (FIC1). The resulting lack of hemimembrane symmetry destabilises the membrane, a phenomenon associated with decreased expression of CEs, including GGT [28, 29]. Without GGT expression, bile salts cannot excrete GGT into the bile, from where it refluxes into plasma (with its activity measured in serum). Others have mutations in *ABCB11*, encoding BSEP, which transfers amino-

acid conjugates of bile acids from cytoplasm of hepatocytes into the canalicular lumen [21]. In this setting BSEP expression may be deficient, whilst expression of other CTPs and of CEs is intact. Consequently, due to the lack of bile salts GGT cannot be leached into bile. The same holds for disorders of bile acid synthesis or conjugation, in which substrates suitable for BSEP are not produced or lack hydrophilicity sufficient to guard against loss of bile acids from bile through canalicular tight junctions [30-32]; without bile salts in the bile, GGT elution cannot proceed [33]. Finally, disorders of intracellular trafficking may lead to abnormal expression of CEs and CTPs alike, a setting in which deficiency in both GGT and BSEP expression as well as in BSEP function likely contribute to failure of serum GGT activity to increase [28, 34, 35]. The morphological appearances of the biopsy specimens in our study were more typical of those seen in FIC1 disease in the form of giant cell hepatocyte change. The morphological appearances in the biopsy specimens and reduction in intensity of staining of both GGT and BSEP seen in this cohort suggest a combination of pathophysiological mechanisms affecting both expression of CE/CTP, membrane stability and bile acid homeostasis contributing to this phenotype rather than the phenotype being secondary to a single underlying CE/CTP defect.

We investigated expression of CTPs and CEs, including GGT, in the liver after observing low or normal serum levels of GGT activity in more than half of our study cohort with clinically documented HP. Indeed, immunohistochemical analysis suggested deficiency of hepatocellular expression of some CTPs, in particular BSEP. Transient intrahepatic cholestasis in a newborn infant has been reported in association with heterozygosity for a deletion affecting the *ABCB11* locus, identified by fluorescence *in situ* hybridization analysis [36]. In our patient cohort, no *ABCB11* mutations were found in the six patients analysed (among ten biopsied).

Our observation could indicate that corticosteroid and thyroxin sufficiency is important for bile flow at this age. Indeed, the histological features observed in this series are reminiscent of, albeit milder

than, the findings in the liver biopsy specimens at presentation from children with mutation-proven *ABCB11* disease and immunohistochemically demonstrated BSEP deficiency. Expression of CD13, serving as a control marker, was robust in all specimens, identifying canaliculi along with cholangiocyte apices throughout the biopsy material.

Shortcomings of our study were limited number of infants who had liver biopsy, as often this procedure is not strongly indicated in presence of confirmed HP and SOD. In addition, not all biopsied patients had stored DNA for retrospective mutational analysis. Finally, recent studies have revealed additional genetic causes of low GGT cholestasis, such as tight junction protein 2 (TJP2) deficiency [37] and myosin 5B (MYO5B) deficiency [37], which were not tested for in this analysis due to lack of DNA.

A mechanism linking cholestasis with adrenal and thyroid insufficiency in HP remains uncertain. Isolated primary hypothyroidism is typically associated with unconjugated hyperbilirubinaemia [38], suggesting that deficiency not of thyroid hormones but rather of corticosteroid hormones could be responsible for the conjugated jaundice observed in primary HP. In normal liver, corticosteroids could enhance bile output by increasing the bile-salt independent fraction of bile flow [39], probably inducing an increase in Na^+/H^+ exchanger (NHE isoform) and $Cl^-/HCO3^-$ exchanger (AE2 member) activity resulting in an increase in bicarbonate excretion [40]. Corticosteroids have been empirically used to improve cholestasis in a number of cholestatic conditions [41-45]. For example, low-dose prednisolone (2 mg/kg/d) improves cholestasis in younger infants with biliary atresia following hepatic portoenterostomy, but with no discernible effect upon eventual outcome [41, 46]. Whether the beneficial effects of the steroids on cholestasis could be related to their inducing expression of CTPs or CEs at bile canaliculi remains speculative.

Our study suggests that endocrine mechanisms could be involved in aberrant expression of CTPs during prolonged neonatal cholestasis associated with primary HP. This observation may have some

implications for other cholestatic conditions and warrants further study using more sophisticated molecular methods for assessment of expression of CTPs in a larger number of patients.

Figure 1: **A:** Liver biopsy demonstrating a giant cell hepatitis with canalicular cholestasis (Patient 2, H&E x200 magnification, scale bar = 50 micrometers) The inset shows an age matched control with alpha-1 antitrypsin deficiency, PiZZ (DPAS stain x400 magnification, black arrows indicating the DPAS positive globules in periportal hepatocytes, scale bar = 20 micrometers). **B:** Immunostaining with CD13 (Patient 2, main image x200 magnification, scale bar = 50 micrometers) demonstrated maintained canalicular CD13 expression, comparable with that seen in periportal hepatocytes in an age matched patient with alpha-1 antitrypsin deficiency (upper inset, x400 magnification, scale bar = 20 micrometers) and in an age matched normal control (lower inset x400 magnification, scale bar = 20 micrometers). **C:** MRP2 immunostaining (Patient 2, MRP2, main image x200 magnification, scale bar = 50 micrometers) demonstrated maintained canalicular expression, comparable with that seen in periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency control (upper inset x400 magnification, scale bar = 20 micrometers) and normal age matched controls (lower inset, x400 magnification, scale bar = 20 micrometers). **D:** There was markedly reduced expression of GGT (Patient 2 main image x200 magnification, scale bar = 50 micrometers) compared with periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency patient (upper inset x400 magnification, scale bar = 20 micrometers) and in age matched controls (lower inset, GGT x400 magnification, scale bar = 20 micrometers). **E:** Only focal punctate canalicular expression of BSEP was seen (Patient 2, main image**,** BSEP with arrows indicating BSEP expression, x400 magnification, scale bar = 20 micrometers) compared with periportal hepatocytes in an age matched patient with alpha-1 antitrypsin deficiency (upper inset x400 magnification, scale bar = 20 micrometers) and age matched controls (lower inset, BSEP x400 magnification, scale bar = 20

micrometers). **F:** Focal punctate canalicular MDR3 expression was also seen (Patient 2, MDR3, main image, arrows indicate canalicular expression, x400 magnification, scale bar = 20 micrometers) compared with periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency control (upper inset, MDR3 x400 magnification, scale bar = 20 micrometers) and in normal controls (lower inset, MDR3 x 400 magnification, scale bar = 20 micrometers).

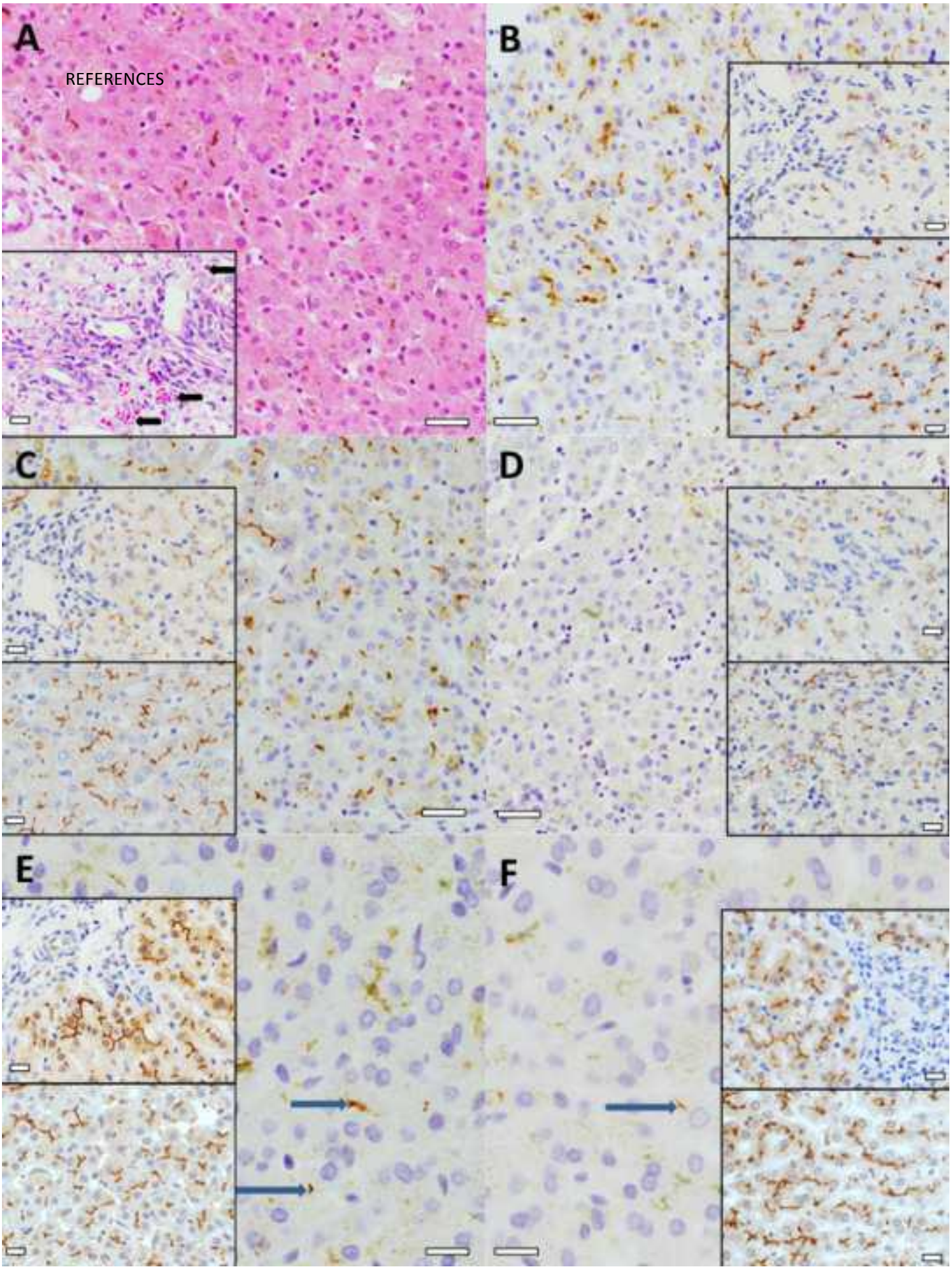
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Figure 1: **A:** Liver biopsy demonstrating a giant cell hepatitis with canalicular cholestasis (Patient 2, H&E x200 magnification, scale bar = 50 micrometers) The inset shows an age matched control with alpha-1 antitrypsin deficiency, PiZZ (DPAS stain x400 magnification, black arrows indicating the DPAS positive globules in periportal hepatocytes, scale bar = 20 micrometers). **B:** Immunostaining with CD13 (Patient 2, main image x200 magnification, scale bar = 50 micrometers) demonstrated maintained canalicular CD13 expression, comparable with that seen in periportal hepatocytes in an age matched patient with alpha-1 antitrypsin deficiency (upper inset, x400 magnification, scale bar = 20 micrometers) and in an age matched normal control (lower inset x400 magnification, scale bar = 20 micrometers). **C:** MRP2 immunostaining (Patient 2, MRP2, main image x200 magnification, scale bar = 50 micrometers) demonstrated maintained canalicular expression, comparable with that seen in periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency control (upper inset x400 magnification, scale bar = 20 micrometers) and normal age matched controls (lower inset, x400 magnification, scale bar = 20 micrometers). **D:** There was markedly reduced expression of GGT (Patient 2 main image x200 magnification, scale bar = 50 micrometers) compared with periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency patient (upper inset x400 magnification, scale bar = 20 micrometers) and in age matched controls (lower inset, GGT x400 magnification, scale bar = 20 micrometers). **E:** Only focal punctate canalicular expression of BSEP was seen (Patient 2, main image**,** BSEP with arrows indicating BSEP expression, x400 magnification, scale bar = 20 micrometers) compared with periportal hepatocytes in an age matched patient with alpha-1 antitrypsin deficiency (upper inset x400 magnification, scale bar = 20 micrometers) and age matched controls (lower inset, BSEP x400 magnification, scale bar = 20 micrometers). **F:** Focal punctate canalicular MDR3 expression was also seen (Patient 2, MDR3, main image, arrows indicate canalicular expression, x400 magnification, scale bar = 20 micrometers) compared with periportal hepatocytes in an age matched alpha-1 antitrypsin deficiency control (upper inset, MDR3 x400 magnification, scale bar = 20 micrometers) and in normal controls (lower inset, MDR3 x 400 magnification, scale bar = 20 micrometers).

Patient	Cholestasis	Giant cell transformation	Fibrosis	Portal inflammation	GGT decreased immunostaining	CD13 decreased immunostaining	BSEP decreased immunostaining	MDR3 decreased immunostaining	MRP2 decreased immunostaining
1	+	-	-	+	+	-	+	+	-
2	+	+	-	+	+	-	+	+	-
3	+	-	-	-	n/a	-	+	+	-
4	+	+	+	+	_	-	+	+	-
5	+	+	-	+	n/a	-	+	-	+
6	+	+	-	+	-	-	+	+	-
7	+	-	-	+	n/a	-	+	+	-
8	+	-	+	-	+	-	+	-	+
9	+	+	+	+	+	-	+	+	-
10	+	+	+	+	+	n/a	+	-	-

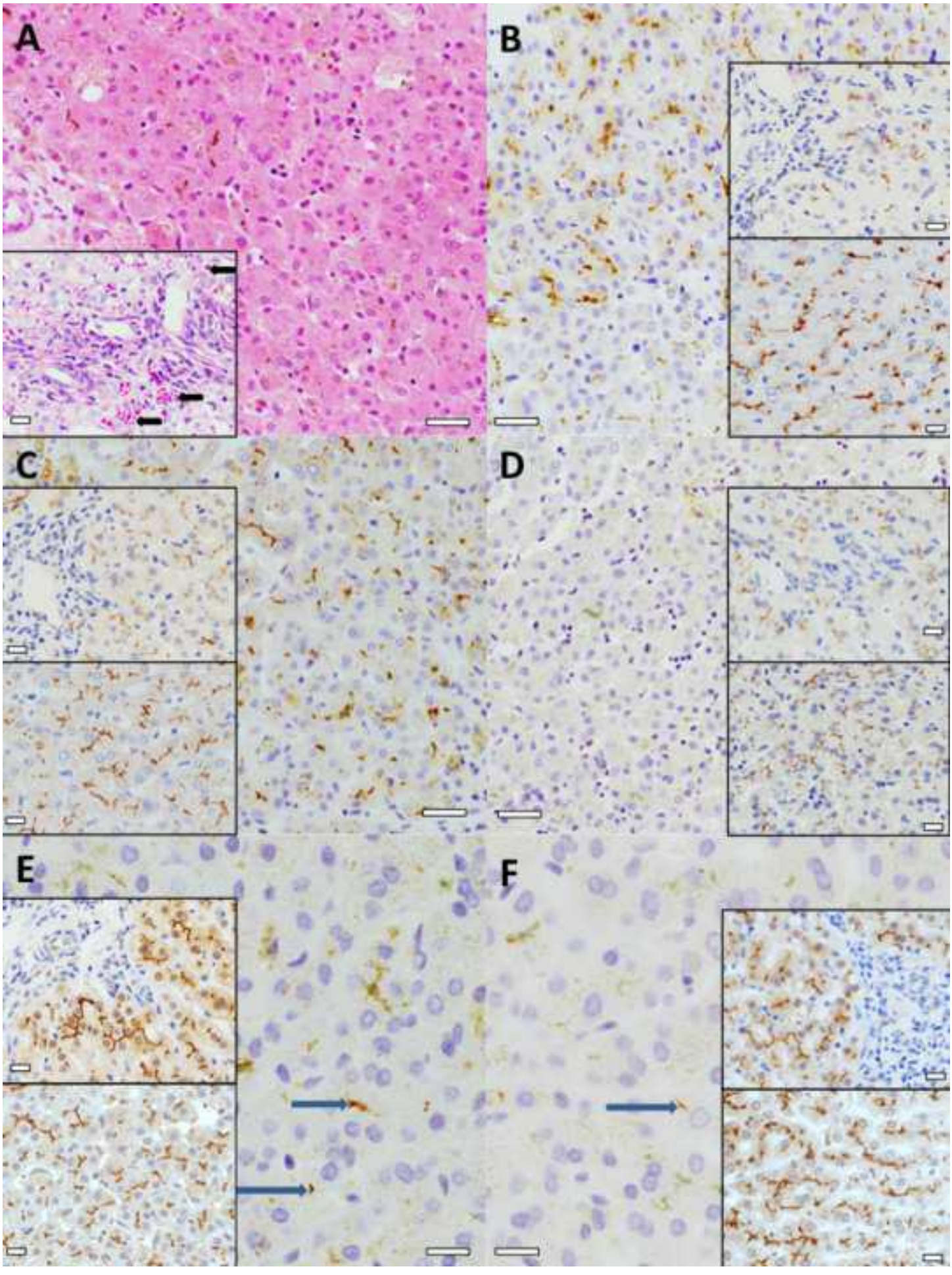
Table 1: Liver histology findings and immunostaining results from ten patients who underwent liver biopsy with evident cholestasis and variable expression of canalicular ectoenzymes and transport proteins. GGT; γ-glutamyltransferase, CD13; alanyl aminopeptidase, BSEP; bile salt export pump, MRP2; multidrug resistance protein 2, MDR3; multidrug resistance protein 3, n/a; non available.

Patient	Bilirubin Total (µmol/L)	Bilirubin Conjugated (µmol/L)	γ-GT (IU/L)	AST (IU/L)
1	212	139	28	91
2	139	111	137	177
3	175	135	56	143
4	74	47	42	91
5	45	36	142	297
6	100	80	282	154
7	287	165	63	156
8	226	127	25	201
9	110	83	154	103
10	213	101	38	455
11	116	88	77	119
12	125	115	95	342
13	99	77	707	130
14	75	56	85	66
15	145	67	63	360
16	54	32	167	93
17	78	43	265	134
18	167	132	234	201
19	79	44	114	176
20	105	48	76	83
21	151	123	259	131

Table 2: Liver biochemical profile of children presenting with conjugated hyperbilirubinaemia and subsequently diagnosed with congenital hypopituitarism. AST; aspartate aminotransferase, γ-GT; γ-glutamyltransferase.

Patient	Age at presentation (weeks)	Sex	TSH (0.3-6 mU/L)	Free Thyroxine (10-26 pmol/L)	Cortisol (nmol/L)	Abnormal Short ACTH	GH deficiency	MRI Head	SOD
1	4	M	6.3	8.2	64	+	+	Ectopic	+
								pituitary	
2	20	F	5.6	8.3	18	+	+	H*	-
3	4	M	2.46	14.1	20	+	+	H*	-
4	6	F	4.21	12	18	+	+	H*	+
5	16	F	<0.1	26.9	44	+	+	n/a	-
6	12	M	8.65	8.5	98	+	-	H*	+
7	3	F	<0.1	9.3	240	+	+	H*	-
8	4	F	1.71	9.8	56	+	-	H*	-
9	12	M	6.59	8.0	31	+	+	H*	+
10	2	M	9.24	21.9	211	+	-	Normal	+
11	4	M	<0.1	22.2	64	+	+	H*	-
12	8	M	7.02	9.7	<30	+	+	n/a	-
13	13	M	3.16	9.1	<20	+	+	H*	+
14	12	F	6.36	6.96	23	+	-	H*	-
15	7	F	2.4	24.3	86	+	+	H*	-
16	6	M	0.79	20.1	51	+	+	H*	+
17	9	M	4.99	8.6	108	+	-	H*	+
18	15	F	<0.1	16.3	67	+	-	H*	+
19	12	M	7.2	9.0	45	+	-	H*	+
20	10	M	<0.1	6.8	34	+	+	H*	-
21	4	F	8.02	16.2	123	+	+	H*	+

Table 3: Demographic, radiological and endocrine data on patients presenting with jaundice and diagnosed with congenital hypopituitarism. TSH; thyrotrophic stimulating hormone, ACTH; adrenocorticotropic hormone, GH; growth hormone, H*; Hypopituitarism, SOD; septo-optic dysplasia, n/a; non available.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